

## A SIMPLE MASS BALANCE MODEL OF NITROGEN FLOW IN A BIOREGENERATIVE LIFE SUPPORT SYSTEM

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A mathematical model of the nitrogen cycle in a bioregenerative life support system (BLSS) was developed to help conceptualize and quantify nitrogen flux and storage in BLSS processes and subsystems. The mathematical model was initially designed as a simple mass balance, donor-controlled system that quantified the amount of nitrogen in moles per person. Dynamic equations were then applied to describe certain relationships more accurately. Comparison of nitrogen fluxes suggests that even at very low atmospheric leakage rates, loss of nitrogen gas would account for the largest nitrogen movement in the "closed" system. This observation decreases the relative importance of denitrification and nitrification in closed system nitrogen balances. Sensitivity analysis was used to determine the relative stability of various model subsystems, and demonstrated the importance of plant nitrogen uptake on overall system dynamics of nitrogen.

Nitrogen Modeling	Advanced life support (ALS)	Bioregenerative life support system (BLSS)
Closed systems	Controlled ecological life support system (CELSS)	

### INTRODUCTION

Bioregenerative life support will be an important tool in the establishment of a permanent manned presence in space. A bioregenerative life support system (BLSS) would function as a self-contained ecosystem, providing food, atmospheric regeneration, and recycled water for human needs. Closure of the BLSS would require a balance in the cycling of all material elements in the system. Nitrogen is important in biological systems as a component of biological molecules (e.g., proteins, nucleic acids). In addition, nitrogen undergoes several biologically mediated transformations in oxidation state. It is cycled among biologically available

and unavailable inorganic molecules, as well as nitrogen-containing organic molecules.

This work describes the development and preliminary testing of a simple, mass balance model of nitrogen cycling within a bioregenerative life support system. This model will be useful for 1) conceptualizing the major reservoirs and fluxes of nitrogen within the system, 2) developing a preliminary understanding of important subsystems, and 3) identifying areas where further research is necessary to better define flux and reservoir estimates. These analyses will lead to the identification of critical areas requiring control mechanisms (i.e., those that most greatly affect overall nitrogen flux within the system).

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## METHODS

*The Model Structure*

The model simulates the nitrogen cycle of a fully closed bioregenerative life support system based on 1) plant growth for food production, atmospheric regeneration, and water purification; 2) aerobic microbial processing of wastes (both inedible plant material and human waste); and 3) secondary food production based on aquaculture.

The entire model was scaled to a one-person level. The model was designed as a steady-state mass balance model (i.e., the net flux for each stock of nitrogen was set to zero). Accordingly, all flux values were determined as moles of nitrogen per person per day, whereas stock, or reservoir, values (the amount of nitrogen residing in the system components) were determined as moles of nitrogen per person. The majority of

fluxes in the dynamic model were based on simple donor-controlled equations. These linear equations equaled the donor stock multiplied by a coefficient; the ratio of the steady-state flux divided by the steady-state donor stock. Exceptions were made for several fluxes to allow the model to act more realistically. The model diagram is presented with the steady-state values in Figure 1, where arrows represent the fluxes and boxes represent the stocks.

Biological transformations in the valence state of nitrogen are accounted for in the model. The ratio of ammonium and nitrate ions generated from the aerobic bioreactor and aquaculture subsystems is controlled by the nitrification reactor prior to introduction into the hydroponic subsystem. Nitrifying microorganisms contained in this bioreactor are chemoautotrophs, which convert ammonium to nitrate as a source of energy. The model also accounts for denitrification by microorgan-

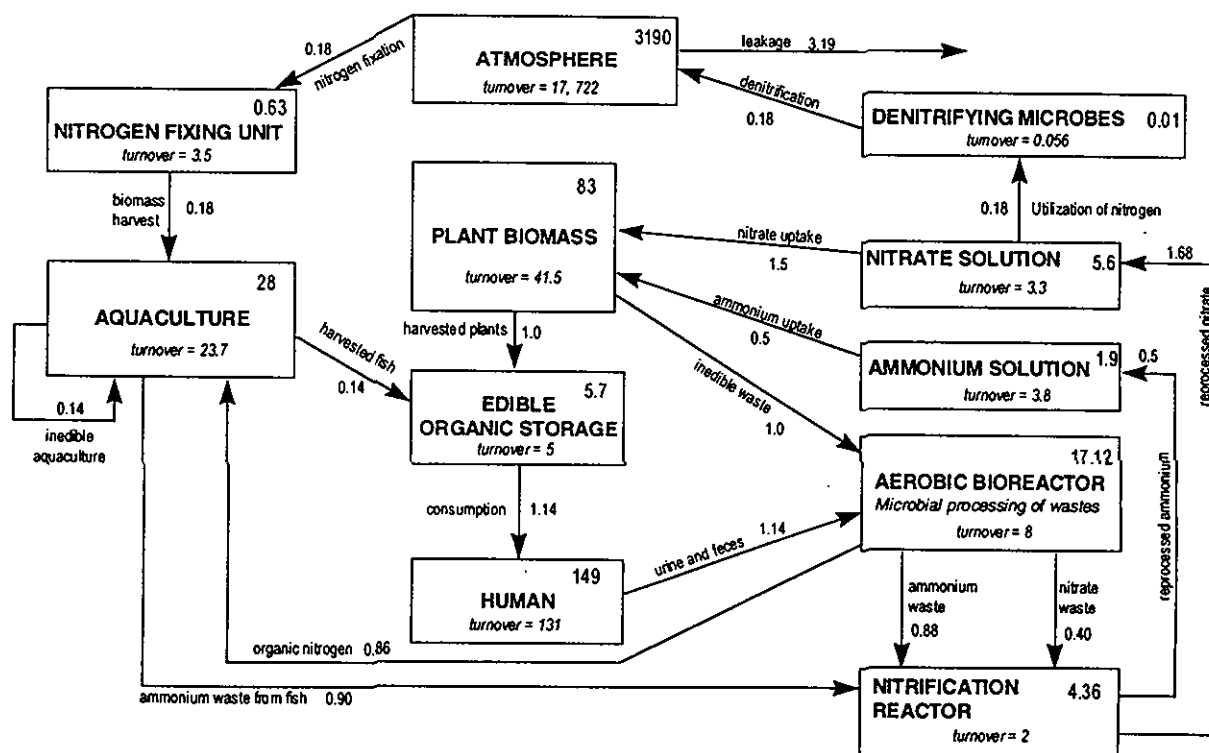


Figure 1. Schematic representation of the initial mathematical model of the nitrogen cycle in a closed bioregenerative life support system. The arrows represent the fluxes and the objects represent the reservoirs. The numbers associated with each represent the initial values. Flux values are presented in moles of nitrogen per person per day, whereas reservoir values are presented in moles of nitrogen per person. Turnover rates are represented in days (italic type).

isms in the root zone (or rhizosphere) of the plants. Denitrification is the conversion of nitrate to nitrogen gas by certain heterotrophic microorganisms that use nitrate as a terminal electron acceptor under microaerophilic or anaerobic conditions. A nitrogen-fixing system based on the conversion of nitrogen gas to organic nitrogen through the action of selected microorganisms is also included in the model.

#### *Source for Model Variables*

The quantity of gaseous nitrogen in the atmosphere was calculated based on a total volume of  $100 \text{ m}^3$ , approximately the same as that of the Biomass Production Chamber (BPC), a prototype, large-scale plant growth facility for BLSS research at Kennedy Space Center (KSC). Present studies indicate that the BPC can provide 310 g per day of edible biomass in  $20 \text{ m}^2$  area (28) or approximately half the quantity of food required for a typical person (30). It is reasonable to assume that the size of a one-person bioregenerative system is the current size of the BPC, given the potential for increases in plant productivity and more efficient space utilization.

Utilizing standard Earth pressure and the mole fraction of nitrogen in the Earth's atmosphere, atmospheric nitrogen amounted to 3190 mol per person. Atmospheric leakage (through seals in construction, extravehicular activities, and even through bulkheads) was 2.8% volume per day on the Apollo 15 spacecraft (3), and 2.6% volume per day on the Space Shuttles (24). Leakage in this closed, bioregenerative model was optimistically estimated at 0.1% per day, resulting in a flux value of 3.19 mol of nitrogen per day per person. Leakage was the only flux in the model that created an imbalance in a stock. However, the assumption is made that this loss will be compensated for by periodic resupply of the facility.

The reservoir and fluxes associated with the human stock were based on a 70-kg individual. Nitrogen content of 149 mol per person was calculated based on Forbes et al. (8). The daily flux of nitrogen in human waste was estimated at 1.14 mol (4,19,31). Assuming that the model human is a healthy adult and ignoring the minor epidermal losses, consumption was considered equivalent to the waste flux. The equation for consumption was adjusted to represent more accurately the needs of a real human. Because a human would conserve food when harvests and supplies are low, a con-

ditional equation was applied to enable less consumption when food stocks fell below a critical storage level, considered to be five times normal consumption or 5.7 mol. Consumption was normally 1.14 mol except when this condition applied; at this point a donor-controlled equation was employed, at 20% of available stores.

Though a human body works to maintain a homeostatic nitrogen content through reabsorption in the kidneys, under starvation conditions protein will eventually be degraded and more nitrogen excreted than consumed (10). For the purpose of this simplistic model, we assumed homeostatic conditions and set the equation for the waste flux equal to the consumption flux.

The *Tilapia*-based aquaculture subsystem receives its nitrogen from three sources: processed wastes, algae from the nitrogen-fixing unit, and fish waste. Recent studies have shown that the aerobic microbial processing of plant and human waste would provide 0.86 mol of organic nitrogen per person per day to an aquaculture subsystem (15). Production of cyanobacteria from the nitrogen-fixing unit (see below) would provide 0.18 mol per day as feed to the *Tilapia*. Approximately half of the *Tilapia* production would be nonfilet biomass (15), and this mass (0.14 mol of nitrogen per day) could be recycled as fish feed. Ammonium waste was determined by taking the difference between inputs and other outputs to this subsystem: 0.9 mol per person per day. The nitrogen reservoir within the aquaculture subsystem was calculated by assuming a daily harvest of 0.1% of the stock and a 100-day growth period: 28 mol of nitrogen per person. This reservoir sizing is an assumption, but was made due to the lack of relevant information specific to this production subsystem.

Production of nitrogen in edible plant biomass (1.0 mol per day) was based on the difference between total human consumption (1.14 mol per day) and the edible fish production (0.14 mol per day). Nitrogen content of total harvested plant biomass was based on a simple mixed diet of candidate ALS crops (four parts potato, two parts wheat, two parts soybean, one part lettuce), and an estimate of biomass production (28), and nitrogen content of these crops (14,26,27) from baseline studies at KSC. The difference between total harvest and edible portion gave an inedible daily plant nitrogen harvest of 1.0 mol. The total nitrogen content within the plant growth subsystem (83 mol per person) was estimated from growth curves of the different crops (29), and assuming that 1) nitrogen content in the plant tissue does not differ with age, and 2) continuous produc-

tion of the crops. An edible organic stock (food storage) was set at five times the consumption flux, to provide an emergency buffer without imposing on the limited space.

The nutrient solution was modeled after current BPC experimental hydroponic solutions, containing a concentration of 7.5 mM of nitrogen (12). In this case, a 3:1 (5.6 to 1.9 mM) nitrate to ammonium ratio was chosen as ideal for the plants, based on its pH balancing effect (11). The BPC uses approximately 900 liters for its 20 m<sup>2</sup> growing area (25). A smaller volume per unit growing area has been suggested to reduce overall system mass (6). Given the previous assumption of increased space utilization for growing area in the BPC we used an average value of 1000 liters to approximate the solution volume.

Plant uptake of nitrogen would equal the output at steady state (i.e., 2.0 mol of nitrogen per person per day). Assuming a ratio of nitrate to ammonium uptake of 3:1, a nitrate uptake value of 1.5 mol per day and an ammonium value of 0.5 mol per day were estimated for the steady-state model.

The Michaelis-Menten equation, which has been known to describe enzyme reaction kinetics as a function of substrate concentration, was applied to this model:

$$V = V_{\max} \frac{[S]}{[S] + K_m} \quad (1)$$

where  $V$  is the reaction rate,  $V_{\max}$  is the maximum reaction rate,  $K_m$  is the substrate concentration at half the maximum reaction rate, and  $[S]$  is the substrate concentration (22). This equation can also be used to describe nutrient uptake by roots (18).

Substrate concentration  $[S]$ , for both ammonium and nitrate, was simply the number of moles of ammonium or nitrate in their dynamic stocks divided by the total volume of hydroponic solution (set at 1000 liters); this determines the molarity of the solution.

The value of  $K_m$  can vary considerably with nutritional status or plant age (13), and Sands and Smethurst (18) list a wide range of  $K_m$  values for various species. We averaged the values from Sands and Smethurst (18) and chose a  $K_m$  of 0.0001 M. We also applied a range test for  $K_m$ , using 0.00001 M and 0.001 M to study the importance of this value.

The maximum reaction rate,  $V_{\max}$ , was represented by a constant, ( $V_{\max}$  when using steady-state values di-

vided by the steady-state value of plant biomass), multiplied by the dynamic plant biomass stock. This equation allowed uptake to be based on the actual amount of biomass present.

$V_{\max}$  steady-state equations:

$$V_{\max}(\text{NO}_3) = \frac{1.5}{0.0056/(0.0056+0.0001)} \quad (2)$$

$$V_{\max}(\text{NH}_4) = \frac{0.5}{0.0019/(0.0019+0.0001)} \quad (3)$$

$$\text{Nitrate (or Ammonia) Constant} = \frac{V_{\max}(\text{NO}_3 \text{ or } \text{NH}_4)}{83} \quad (4)$$

$$\begin{aligned} \text{Uptake } (\text{NO}_3 \text{ or } \text{NH}_4) = \\ \text{NO}_3 \text{ (or } \text{NH}_4) \text{ Constant} \times \text{Plant Biomass} \times \\ \frac{[S]\text{NO}_3 \text{ (or } \text{NH}_4)}{[S]\text{NO}_3 \text{ (or } \text{NH}_4) + K_m} \end{aligned} \quad (5)$$

Denitrification was modeled as a function of nitrate uptake rather than nitrate concentration because: 1) our empirical data on denitrification were based on plant nitrate uptake (23), 2) root activity (i.e., nitrate uptake) affects carbon and oxygen availability to the denitrifying microbes, and 3) the nitrate concentration at steady state is three orders of magnitude greater than the half-saturation constant for denitrification (20). Given a nitrate uptake of 1.5 mol of nitrogen per day, the 12% rate of denitrification (23) would equal 0.18 mol per day. A separate stock to represent the microorganisms inhabiting the rhizosphere zones of the roots was utilized for easier examination of components during the sensitivity analysis. Stock size was estimated from total bacterial numbers reported for the BPC of  $1.6 \times 10^{14}$  cells (9). This cell number was converted to cell volumes using an estimate of  $0.1 \mu\text{m}^3$  per cell (1,17). Total biovolume was converted to nitrogen using a conversion factor of  $2.5 \times 10^{-14}$  g of nitrogen per  $\mu\text{m}^3$  (16). This gave a final value of 0.4 g or 0.028 mol of nitrogen in the rhizosphere microflora. We estimated that 40% of the total microbes were denitrifiers, yielding a steady-state stock of 0.01 mol nitrogen in the denitrifier pool. Denitrification from the bioreactors in resource recovery was not included due to lack of specific information and to maintain model simplicity. The necessity of including this flux in the model will be discussed later.

Biological nitrogen fixation could be used to convert  $N_2$  to biologically available nitrogen. In our steady-state model, we assumed that nitrogen fixation would equal the estimate of denitrification. There are different thoughts as to what organism might be used as a nitrogen fixer in a BLSS [e.g., *Rhizobia* bacteria in symbiosis with plants (legumes), cyanobacteria alone, or a cyanobacteria growing in symbiosis with a water fern]. Doubling time ranges from 1.5 to 9.1 days for two such organisms utilizing various bioreactors designs for a BLSS (2,5). We approximated the doubling time for our model as an average for the two types of organisms: 3.5 days. An adequate nitrogen biomass for the nitrogen-fixing unit would therefore be 0.63 mol per person, 3.5 times the required daily fixation. The nitrogen fixation equation was made recipient controlled, as it is more dependent on its amount of biomass than on the amount of nitrogen in the atmosphere, which is nonlimiting.

The aerobic bioreactor for microbial processing of wastes receives inputs from human waste and inedible biomass, resulting in a total flux of 2.14 mol of nitrogen per person per day. Based on an 8-day turnover of material (7), the standing stock of the aerobic bioreactor would be 17.12 mol. Nitrogen would be removed from the bioreactor as ammonium, nitrate, and organic nitrogen. The bioreactor would provide 0.86 mol of organic nitrogen per day to the aquaculture subsystem (see above). Nitrate output was based on inedible plant biomass analyses (14) and estimated at 0.4 mol of nitrogen per person per day. The final difference determines the ammonium waste of 0.88 mol per day.

A nitrification reactor is used to adjust the ratio of nitrate to ammonium input to the hydroponic solution based on plant uptake rates. The stock was set equal to 4.36 mol based on a 2-day turnover (21).

#### *Sensitivity Analysis*

A sensitivity analysis was conducted on the model to conceptualize which stocks would be affected most by abnormalities and which fluxes are the most influential in the system. The model was run at steady state for 240 days to attain a baseline. This time frame is a reasonable scenario that allows for atmospheric resupply of nitrogen from Earth when needed (to correct for leakage imbalance), as well as allowing parameters to reach possible equilibria or demonstrate their fluctuations. Each flux equation was increased or decreased

independently, by 10% from its initial steady-state value and run for the same period of time. Percentage change of all stocks and fluxes from steady state was noted at the end of 240 days.

#### *Materials and Apparatus*

The mathematical model was constructed using the Macintosh version of *ithink*™ 2.2.1 software. All integration was performed using the fourth-order Runge-Kutta numerical method. Calculations were made numerically every 0.05 days to simulate continuous flow of nitrogen fluxes.

## RESULTS AND DISCUSSION

#### *Conceptualization of the Model*

One of the purposes of the model was to provide an initial quantification of a closed bioregenerative life support system. The relative amounts of nitrogen contained in each of the stocks ranged over six orders of magnitude, from the small denitrifying microbe stock to the large atmospheric stock (see Fig. 1), which is two orders of magnitudes greater than any other stock. Turnover rates of the different stocks (stock divided by flux) ranged from 17,722 days for the atmosphere to 0.056 days for the denitrifying microbes (Fig. 1).

Our optimistic, gaseous nitrogen leakage estimate of 0.1% per day still yielded the largest flux in the model. Nitrogen loss through leakage could be reduced by decreasing total atmospheric pressure or by reducing the partial pressure of  $N_2$  by replacement with another inert gas.  $N_2$  could be resupplied directly (i.e., gaseous additions) or indirectly as nitrogen salts or stored food and reliance on concomitant denitrification (i.e., encourage denitrification rather than discourage). However, because the estimated rates of denitrification are so much lower than the estimated leakage rates, direct resupply of  $N_2$  gas appears to be necessary.

#### *Sensitivity Analysis*

The results of individually raising or decreasing each flux by 10% are presented in Tables 1 and 2, respectively. The exception is for  $K_m$ , which was changed by a factor of 10 in each instance. These tables indicate the percent deviation in the stocks from their steady state after 240 days. This time period in no means showed maximum deviation or a steady state, but did

Table 2. Results of the Sensitivity Analysis Across the Stocks With a Decrease in Individual Fluxes by 10%

Decreased Flux	Affected Stock										
	Aerobic Bioreactor	Ammonium Solution	Aquaculture	Atmosphere	Denitrifying Microbes	Edible Organic Storage	Human	Nitrate Solution	Nitrification Reactor	Nitrogen Fixation Unit	Plant Biomass
Ammonium uptake	-7.73	487.97	-5.83	-0.07	-7.56	-7.73	0.00	54.36	-6.90	0.00	-8.13
Ammonium waste	3.45	1.43	2.87	-0.01	-1.09	-0.58	0.00	-1.39	-0.96	0.00	-1.06
Ammonium waste from fish	-1.13	3.81	8.52	-0.02	-1.80	-0.54	0.00	0.04	-1.63	0.00	-1.81
Biomass harvest	4.81	21952.64	1754.38	-100.00	12.82	5307.43	0.00	25197.98	784.63	59.46	10.85
Consumption	-9.28	-10.80	-7.41	-0.09	-9.31	272.78	0.00	-30.69	-8.49	0.00	-8.61
Denitrification	0.00	0.00	0.00	0.00	11.11	0.00	0.00	0.00	0.00	0.00	0.00
Harvested fish	-0.64	0.04	0.83	0.00	-0.04	-1.17	0.00	-0.13	-0.04	0.00	-0.04
Harvested plants	0.46	-51.41	0.40	0.02	0.44	-4.18	0.00	-75.09	0.44	0.00	5.75
Human wastes	-16.96	-35.74	-12.75	-0.12	-15.23	-12.60	17.39	-61.12	-15.12	0.00	-12.90
Inedible waste	-3.96	-52.58	-3.18	-0.03	-3.67	112.14	0.00	-75.88	-3.63	0.00	1.65
$K_m$ 0.00001	0.36	-90.60	0.43	0.03	0.34	90.61	0.00	-91.65	0.39	0.00	0.69
Leakage	0.00	0.00	0.00	2.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitrate uptake	-28.30	151.30	-21.14	-0.37	-35.90	-28.35	0.00	816.28	-25.16	0.00	-29.90
Nitrate waste	1.54	0.61	1.29	-0.01	-0.48	-0.25	0.00	-0.78	-0.42	0.00	-0.47
Nitrogen fixation	-17.27	-52.58	-29.13	1.20	-21.96	-18.53	0.00	-75.99	-21.97	-99.89	-17.63
Organic nitrogen	4.33	-0.51	-5.06	0.01	0.54	-0.18	0.00	1.54	0.45	0.00	0.51
Reprocessed ammonium	-5.41	-59.92	-3.98	-0.03	-4.70	-5.43	0.00	165.69	-2.54	0.00	-5.74
Reprocessed nitrate	-2.11	345.82	-1.46	-0.04	-4.28	-2.14	0.00	-54.09	6.38	0.00	-2.30
Utilizing nitrogen	0.51	-8.92	0.37	-0.14	-8.59	21.44	0.00	32.11	0.45	0.00	1.14

Percent deviation from stock values in steady-state model is noted.

allow time for the model to approach a partial equilibrium where possible. The sensitivity analysis showed that stock values often did not respond in a linear fashion, but would increase and decrease relative to fluctuations in other stocks. The equations allowed for unrealistic increases and decreases of nitrogen to the subsystems. We could have applied control mechanisms (conditional equations) to prevent this but that would have impeded seeing where the majority of change occurs and which are the most influential fluxes. For example, the single conditional equation we used was to maintain food consumption, and that resulted in the human stock being unaffected by the changes in the model. We chose only to apply this type of equation to the human stock as the human is what a BLSS is being designed for and will be the one stock not open to manipulation.

The fluxes connected directly to the nitrogen-fixing unit yielded the most dramatic changes in the model. Increasing nitrogen fixation by only 10% caused a dramatic shift of nitrogen from the atmosphere to the other components of the system (excluding the homeostatic human). Nitrogen fixation vastly exceeded denitrification; hence, the atmosphere quickly empties in just over 210 days. Decreasing the biomass harvested from the nitrogen-fixing unit has the same effect; as more biomass is present to fix nitrogen, the biomass is increased and therefore removes even more nitrogen from the atmosphere. The opposite changes, decreasing nitrogen fixation or increasing the biomass harvested, empties the nitrogen fixation unit in approximately 120 days. These extreme results are due to two reasons: 1) the nitrogen fixation unit utilizes the only true recipient-controlled equation in the model, thereby setting up positive feedback, and 2) the fixation unit can draw on the immense pool of atmospheric nitrogen and direct it to a much smaller pool of organic and ionic-inorganic nitrogen forms. The dramatic results are partially an artifact of the model; the positive feedback loop would be stopped by the vessel in which the cyanobacteria reside. Yet there is a strong possibility that any additional nitrogen added into the small pool of nongaseous nitrogen could be used to control the system, whether by a nitrogen-fixing unit, supply of nitrogen salts, or stored foods. Due to atmospheric leakage creating such a need for gaseous resupply of nitrogen, a nitrogen-fixing unit is unwarranted and the latter two input options more probable as a nitrogen-controlling mechanism in a BLSS.

Changes in plant uptake significantly influenced nitrogen distribution in the system. The largest effects were observed in the stocks in direct contact (food storage, solution concentration), but there is also a 5–35% change in “distant” stocks when decreasing flux (Table 2). As the  $K_m$  value is not well defined, we increased and decreased  $K_m$  by a factor of 10 instead of 0.1, to reflect the range of reported data (18). For the sensitivity analysis, the  $K_m$  used in our  $V_{max}$  constant was not changed; only the  $K_m$  in the actual uptake equation was changed. This adjustment had a relatively large effect on the model, for adjusting  $K_m$  adjusted the influential uptake equations. The large effects of plant uptake on nitrogen dynamics demonstrate that if we cannot define and control uptake adequately, nitrogen buffers will be required.

Other influential systems included fluxes of harvested plants and inedible waste, as these fluxes are central to the entire model. Increasing the plant biomass harvest of edible and inedible material has a negative effect on many subsystems because it decreases the amount of plant biomass and concomitant nitrogen uptake. This results in less nitrogen shunted through all the subsystems, causing an accumulation in the nutrient solutions. In reality, if too many plants were harvested, one would not be able to harvest again until the younger plants reach maturity. For this scenario shown by this sensitivity analysis to be realistic, it would probably imply partial removal of plants due to disease or some other crop failure.

A harvest decrease did not show the same large effects. The plant biomass stock is enlarged whereas the solution concentrations are significantly decreased. New equilibria were reached in approximately 120 days and the data appeared fairly steady. When inedible waste is decreased, it increases the plant biomass stock, which initially increases many systems that the harvest flux directly influences. These effects start to decrease after approximately 30 days, when excess biomass is tied up in the edible storage box. Most stocks decrease to support the edible storage increase, but the analyses do illustrate that a new equilibrium is being approached.

#### *Necessary Reservoir and Flux Research*

Denitrification from the aerobic processing of waste was not included in this model for reasons of simplicity and for lack of specific data. Denitrification from bioreactors is a concern, however, and could be very

high if microaerophilic or anaerobic conditions exist in the reactors. Denitrification in the bioreactor could be considered as a means of resupplying  $N_2$  gas, but because rhizosphere denitrification had little effect, bioreactor denitrification is also unlikely to significantly influence the system.

Accurate modeling of continuous nitrogen dynamics in the plant subsystem requires more complete data on nitrogen accumulation in plant tissue with age. For example, the lack of detailed information on nitrogen distribution throughout plant growth led to the assumption that moles of nitrogen per gram of dry weight were constant throughout the growth cycle.

The stock value chosen for the aquaculture unit was speculative. To assess the need for an accurate value for the model, the stock itself was increased and decreased by a factor of 10. We repeated the sensitivity analysis utilizing only the most influential fluxes (i.e., the fluxes into and out of the nitrogen-fixing unit). These fluxes were changed by 10% and the results compared to the standard sensitivity analysis. The changes to all stocks from this partial sensitivity analysis merely demonstrated that increasing the amount of nitrogen in the aquaculture unit gives a more adequate buffer to the model, and decreasing, a less adequate buffer. If aquaculture is to be included in a BLSS, it will need to be studied in greater depth to firmly establish its variables. However, because of subsystem complexity, a first-generation BLSS will probably not include aquaculture.

### CONCLUSIONS

Complete bioregenerative life support testbeds will not be finished for several years, and even in such testbeds, specific measurements of individual element cycling will be difficult. A mass balance model conceptualizes the fluxes and stocks of the system and defines areas for further research. The analysis of this model provided several interesting insights.

The model shows that leakage is the largest flux in the system, even when leakage was set to an optimistic value of 0.1%. This leakage was 18 times greater than denitrification (the only flux entering the atmosphere); hence, resupply of nitrogen gas will be needed. Because the rate of nitrogen fixation significantly affected stocks of nitrogen in other subsystems, this subsystem could be used as a controlling mechanism. However, because nitrogen fixation would compound the loss of atmospheric nitrogen, resupply of nitrogen as nitrate

salts or stored food may be a more effective control mechanism.

Variation in the uptake of nitrogen by plants also significantly influenced other stocks of nitrogen. Because it is known that uptake will vary considerably according to crop type, age, and environmental conditions (13), further research is needed to define and control resulting perturbations in the nitrogen balance of the system.

The mass balance approach has increased our understanding of the distribution and cycling of nitrogen within a bioregenerative system. Simple mathematical analysis has helped prioritize areas for further research, both in terms of parameter definition and control system development. Although only biological processes have been discussed in this model, the influence of physical-chemical processes on nitrogen cycling could be readily evaluated with this approach.

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